ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2005, p. 776–778 Vol. 49, No. 2
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.2.776–778.2005

Detection of KPC Carbapenem-Hydrolyzing Enzymes in *Enterobacter* spp. from Brooklyn, New York

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Received 17 May 2004/Returned for modification 10 August 2004/Accepted 3 October 2004

***Enterobacter* spp. are rarely resistant to carbapenems. In this report, one *Enterobacter* sp. isolate possessed bla_{KPC-3} and two possessed bla_{KPC-2}. For all three strains, the imipenem MICs were dependent on the inoculum and testing method; two were reported by the clinical laboratories to be carbapenem susceptible. Improved detection methods will be necessary to identify these enzymes.**

Enterobacter spp. are the third most common pathogen causing pneumonia and the fifth most common pathogen causing bloodstream and surgical site infections in intensive care facilities in the United States (15). Although ~25% are resistant to expanded-spectrum cephalosporins, carbapenem-resistant *Enterobacter* spp. remain very unusual (3). Carbapenem resistance in *E. aerogenes* has been attributed to overproduction of chromosomal cephalosporinase and loss of porins (1, 4, 5, 16). *E. cloacae* rarely may acquire class A carbapenem-hydrolyzing β-lactamases, such as NMC-A (9, 11) and IMI-1 (14). The carbapenem-hydrolyzing enzyme KPC-2 has been recovered in isolates of *Klebsiella* spp. (2, 7, 18) and in an isolate of *E. cloacae* (A. Hossain, M. J. Ferraro, R. M. Pino, R. B. Dew, E. S. Moland, T. J. Lockhart, K. S. Thomson, R. V. Goering, and N. D. Hanson, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-664, 2003). A related enzyme, KPC-3, has been recovered in isolates of *K. pneumoniae* (K. Young, P. Tierno, Jr., L. Tysall, M. F. Palepou, R. Painter, D. Suber, D. Shungu, L. Silver, K. Inglima, J. Kornblum, N. Woodford, and D. Livermore, Abstr. 43rd ICAAC, abstr. C2-50, 2003) and *E. coli* (T. Hong, E. S. Moland, B. Abdalhamid, N. D. Hanson, J. Wang, C. Sloan, D. Fabian, A. Farrajallah, J. Levine, and K. S. Thomson, Abstr. 43rd ICAAC, abstr. C1-665, 2003). In this report, we describe the finding of the β-lactamase KPC-3 in a clinical isolate of *E. cloacae*. The finding of this β-lactamase prompted a retrospective examination of the prevalence of KPC-possessing *Enterobacter* spp. in Brooklyn, N.Y.

A clinical isolate of carbapenem-resistant *E. cloacae*, M61782, was identified in October 2003. The prevalence of KPC β-lactamases was estimated by testing clinical isolates of *Enterobacter* spp. collected during citywide surveillance studies conducted in 2001 and 2003 (13). Identification of KPC-possessing isolates was confirmed by the API 20E system (bioMerieux).

Antibiotics were either obtained from Sigma, Inc. (St. Louis, Mo.) or were gifts from the manufacturers. MICs were determined using the agar dilution method, and they were interpreted according to established methods (8). MICs of tigecycline were determined by the broth microdilution technique. The MICs for KPC-possessing isolates were also tested by the Etest method and the broth microdilution method in cation-supplemented Mueller-Hinton broth using a variety of inocula.

Isoelectric focusing was performed on a polyacrylamide gel (ampholytes [pH 3 to 10]) as previously described (12). β-Lactamases were amplified using previously described primers and conditions (2, 10). Isolates collected during the citywide surveillance studies with a ceftazidime MIC of ≥32 μg/ml were chosen to be tested for the presence of KPC enzymes. Amplified products underwent bidirectional sequencing performed with the automated fluorescent dye terminator sequencing system (Applied Biosystems, Foster City, Calif.). The following additional internal primers were included for sequencing of bla_{KPC}: 5′-AGCTGAACTCCGCCATCC-3′ (forward) and 5′-CCGCCCAACTCCTTCAGC-3′ (reverse).

In October 2003, *E. cloacae* M61782 was repeatedly isolated from a wound culture. The patient resided in a nursing home and had previously been at another hospital. The isolate was found by the clinical laboratory to be resistant to all tested antibiotics except amikacin. The patient was successfully treated with a regimen that included amikacin and gatifloxacin. Isoelectric focusing revealed β-lactamases with pI values of 5.4,

TABLE 1. Susceptibility results of *Enterobacter aerogenes* and *E. cloacae* gathered during citywide surveillances in 2001 and 2003

Drug	% Susceptible			
	E. aerogenes		*E. cloacae*	
	2001 (n = 77)	2003 (n = 43)	2001 (n = 124)	2003 (n = 92)
Ceftriaxone	81	63	66	63
Ceftazidime	69	63	66	70
Cefepime	92	84	84	93
Imipenem	100	98	100	100
Ertapenem	97	95	97	98
Meropenem	99	98	99	100
Piperacillin-tazobactam	74	77	67	64
Tigecycline	ND[a]	98[b]	ND[a]	96[b]
Ciprofloxacin	78	79	82	86
Amikacin	95	88	93	99

[a] ND, not done.
[b] Percent of isolates for which the MIC was ≤2 μg/ml.

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TABLE 2. MICs of carbapenems (in micrograms/milliliter) for three KPC-possessing strains of *Enterobacter* spp.

Isolate	Agar dilution (10^4 CFU/spot)			Etest (imipenem)	Broth microdilution (imipenem)			
	Meropenem	Ertapenem	Imipenem	10^8 CFU/ml	10^4 CFU/ml	10^5 CFU/ml	10^6 CFU/ml	10^7 CFU/ml
M61782	4	16	16	>32	2	4	16	>32
DM303	4	8	4	24	0.5	2	4	>32
MA504	8	8	16	>32	0.5	2	8	>32

6.7, and >9. Sequencing results confirmed the presence of bla_{KPC-3} and bla_{TEM-1}.

A total of 351 single-patient *Enterobacter* spp. isolates were collected in the citywide surveillances conducted in 2001 and 2003, including 120 *E. aerogenes*, 216 *E. cloacae*, and 15 other *Enterobacter* spp. isolates. Susceptibility results are reported in Table 1. A total of 111 ceftazidime-resistant isolates were screened for KPC β-lactamase. All but 1 of the 111 isolates were found to be susceptible to carbapenems according to agar dilution methodology. Two of the 111 isolates were found to harbor KPC enzymes, including an *E. cloacae* isolate (DM303) in 2001 and an *E. aerogenes* isolate (MA504) in 2003. Ribotyping revealed that the *E. cloacae* isolate DM303 was unrelated to M61782 (data not shown). Both isolates possessed a β-lactamase with a pI of 6.7, identified by nucleotide sequencing as bla_{KPC-2}. The isolate of *E. cloacae* originated from a patient with renal failure and continuous bacteremia spanning 20 days. Five of the six blood cultures growing *E. cloacae* were reported by the clinical microbiology laboratory as susceptible to imipenem; the sixth was considered intermediate (using the Dade Microscan WalkAway System; Dade International, Inc., West Sacramento, Calif.). The patient was successfully treated with aminoglycoside therapy and catheter removal. The *E. aerogenes* MA504 isolate, cultured from the sputum of a patient with necrotizing pneumonia, was reported as susceptible to carbapenems by the clinical microbiology laboratory by using the Vitek System (bioMerieux Vitek, Hazelwood, Mo.). The patient was treated with a regimen that included imipenem, but the patient expired after 1 week of therapy.

The imipenem MICs for M61782, DM303, and MA504 were tested using a variety of methods (Table 2). The interpretation of the susceptibility of these strains differed considerably depending on the method and inoculum used. For the broth microdilution method, the imipenem MIC increased considerably as the amount of inoculum increased.

Carbapenems are an important component of antimicrobial regimens for nosocomial infections. Carbapenem resistance in *E. aerogenes* (1, 4, 5, 16) is rarely reported; this is the first report of a carbapenemase in *E. aerogenes* and of bla_{KPC-3} in *E. cloacae*.

The emergence of KPC-type carbapenem-hydrolyzing enzymes in the northeastern United States is alarming. They may reside on transmissible plasmids (6, 17, 18) and have been found in different genera (2, 6, 7, 18). Our retrospective search for *Enterobacter*-possessing KPC β-lactamases suggests that clinical laboratories may not be accurately identifying these isolates. Although our data are limited to only three isolates, two were reported to be susceptible to carbapenems by using automated systems. Compared to the results obtained using the Etest method, the imipenem MICs were considerably lower using agar and broth dilution with standard inocula. Because isolates carrying KPC enzymes are frequently resistant to other classes of antibiotics (2), treatment strategies are severely hampered. Only a concerted effort involving improved detection and infection control will prevent the continued spread of these resistant pathogens.

Nucleotide sequence accession number. The nucleotide sequence of the bla_{KPC-3} gene from *E. cloacae* M61782 has been included in the GenBank database as accession number AY522950.

This work was supported by grants from Merck & Co., Inc.; Pfizer, Inc.; Wyeth-Ayerst Pharmaceuticals; and Elan Pharmaceuticals.

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